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Expression of activated *ras* has been correlated with increased tumor resistance to apoptosis. For example, we have demonstrated that tumors arising in MMTV-*ras* mice display both low spontaneous and chemotherapy-induced apoptosis. The goal of this project is to determine the role of two major Ras effectors, Raf1 and PI3K, in mediating tumor resistance to apoptosis. Transgenic mice expressing either constitutively active or dominant-negative forms of *raf*1 and PI3K are being created and characterized. In parallel, studies in cell cultures from MMTV-*ras* tumors are being performed to determine the effects of specific inhibitors of Raf1 or PI3K on tumor cell growth and apoptosis.

We have generated one line of transgenic mice expressing activated *raf*1, and three lines expressing dominant-negative *raf*1. We have also established cell cultures from MMTV-*ras* tumors, and demonstrated that these cells maintain Ras protein expression and dependence upon Ras function for growth. In the coming year we will focus on characterization of the transgenic mice, and on the pharmacologic effects of Raf1 or PI3K inhibition in tumor cell cultures. These studies should permit us to define the molecular pathways by which Ras confers tumor resistance to apoptosis.

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INTRODUCTION

The Ras proteins control a wide range of cellular properties, both in normal and neoplastic cells, and a large number of effector pathways downstream of Ras are involved in mediating Ras function. One of the cellular properties of enormous clinical importance in the treatment of cancer is the relative susceptibility of tumor cells to the induction of apoptosis. In a variety of cell culture and *in vivo* model systems, activation of Ras has been shown to promote cellular resistance to apoptosis. We have conducted extensive studies on tumors arising in MMTV-v-Ha-*ras* transgenic mice, and have found that these tumors exhibit very low levels of spontaneous apoptosis, and also display marked resistant to chemotherapy-induced apoptosis. Our current goal is therefore to conduct studies in MMTV-*ras* tumor cell cultures and to create additional lines of transgenic mice that will allow us to identify the potential role of two major Ras effector pathways (Raf and PI3K) in mediating Ras-induced apoptotic resistance. The specific aims of this proposal are to:

I. Assess the role of Raf signaling in mammary tumorigenesis in transgenic mice expressing an activated *raf* gene, and in tumor cell cultures from MMTV-*ras* tumors.
II. Similarly assess the role of PI3K in transgenic mice expressing a dominant-negative or constitutively activated PI3K gene, and in tumor cell cultures.
III. Explore the potential role of various members of the *bcl-2* gene family in suppression of apoptosis in tumors and tumor cell cultures from the various classes of transgenic mice.

It is hoped that the results from these studies will contribute to a better understanding of the role of Ras in tumorigenesis, tumor properties, and resistance to therapeutic treatments, and perhaps ultimately to improved strategies for targeting Ras effector pathways in cancer therapy.

BODY - Annual Summary

It should first be pointed out that our research laboratory moved from The Cancer Therapy and Research Center in San Antonio, TX to Virginia Commonwealth University in Richmond, VA, during the past year. This necessitated that all animal breeding related to this project cease for approximately four months (January - April, 2000). In addition, our transgenic mouse colony was scaled back to the bare minimum required for maintenance of lines until the mice were moved to Richmond in May, 2000. In the months since then, we have concentrated on re-establishing a functional research laboratory and re-expanding our animal colony. We are now poised to pick up where we left off on this project prior to our move.

Our efforts thus far have focused on creation of transgenic mice expressing mutant *raf*1 genes. Specifically, we have obtained both a cDNA encoding a constitutively active form of *raf*1 (BXB) and a dominant-negative form (C4B) from the laboratory of Dr. Ulf Rapp, and have generated transgenes with both in which the mutant *raf*1 cDNAs have been placed under the control of the MMTV promoter. Further, we have generated one line of MMTV-BXB transgenic mice, and three lines of MMTV-C4B transgenic mice. Female mice of all lines are fertile, and in general have been able to lactate. We did observed a failure to lactate in the first breeding of the MMTV-BXB founder (a female), but she was able to lactate with subsequent litters. We are therefore investigating whether this will be a common finding in females of this line. Additionally, we have observed a mammary tumor in one MMTV-C4B female, although this tumor subsequently regressed. The appearance of tumors in C4B mice is surprising, since we are expecting the constitutively active, rather than the dominant negative, form of *raf*1 to have oncogenic potential. Thus, it will be of interest to determine whether this is a reproducible finding, and if so, to thoroughly characterize tumors arising in these mice.

We are presently breeding these mice to expand each of the lines. In addition, we will be conducting Northern/RT-PCR analysis in the near future to characterize transgene expression levels in each line.

In addition, we have established a number of early passage epithelioid cell cultures from both MMTV-*ras* and MMTV-*ras*/p53$^{-/-}$ tumors. Cells at passage 5 have been shown to continue to express Ras at high levels by immunohistochemical analysis (Appendix, Fig. 1). Further, we have demonstrated that they retain dependence upon Ras for proliferation, since growth in culture is nearly abolished by the addition of a farnesyltransferase inhibitor, L-744,832 (Appendix, Fig. 2). Thus, these cells should be suitable for use in proposed studies to investigate the effects of inhibitors specific for Raf1 or PI3K.

With regard to training that has been carried out under this grant, the studies performed to date were carried out primarily by two researchers in my lab, David E. Bearss and Mark A. Subler. David Bearss initiated these studies while he was a graduate student in my lab (although he was not supported by this grant). He has subsequently received his Ph.D. degree and is presently doing post-doctoral studies in the laboratory of Dr. Daniel D. Von Hoff at the University of Arizona Cancer Center in Tucson, AZ. Mark Subler is a post-doctoral fellow in my laboratory who moved with me from San Antonio to Richmond. He is in the process of being promoted to position of Instructor in the Department of Human Genetics at VCU, and will be continuing both on this project and on additional studies related to p53 function. I am currently advertising for a new post-doctoral fellow who will devote 100% effort to this project. In addition, I hope to attract a graduate student from the Human Genetics, Biochemistry, or Pharmacology graduate programs in the coming year who would work on some aspect of this project.

KEY RESEARCH ACCOMPLISHMENTS

- construction of MMTV-BXB and MMTV-C4B transgene constructs
- establishment of one line of MMTV-BXB transgenic mice
- establishment of three lines of MMTV-C4B transgenic mice
- establishment of early passage MMTV-*ras* tumor cell cultures

REPORTABLE OUTCOMES

- We are in the early stages of development of the mouse models to be used in the proposed studies. Therefore, no publications have resulted from these studies yet.
- Two new transgenic mouse models (MMTV-BXB and MMTV-C4B) have been developed under this award. Once these mice are characterized and we have published a primary description of them, they will be made readily available to other investigators who request them.

APPENDIX

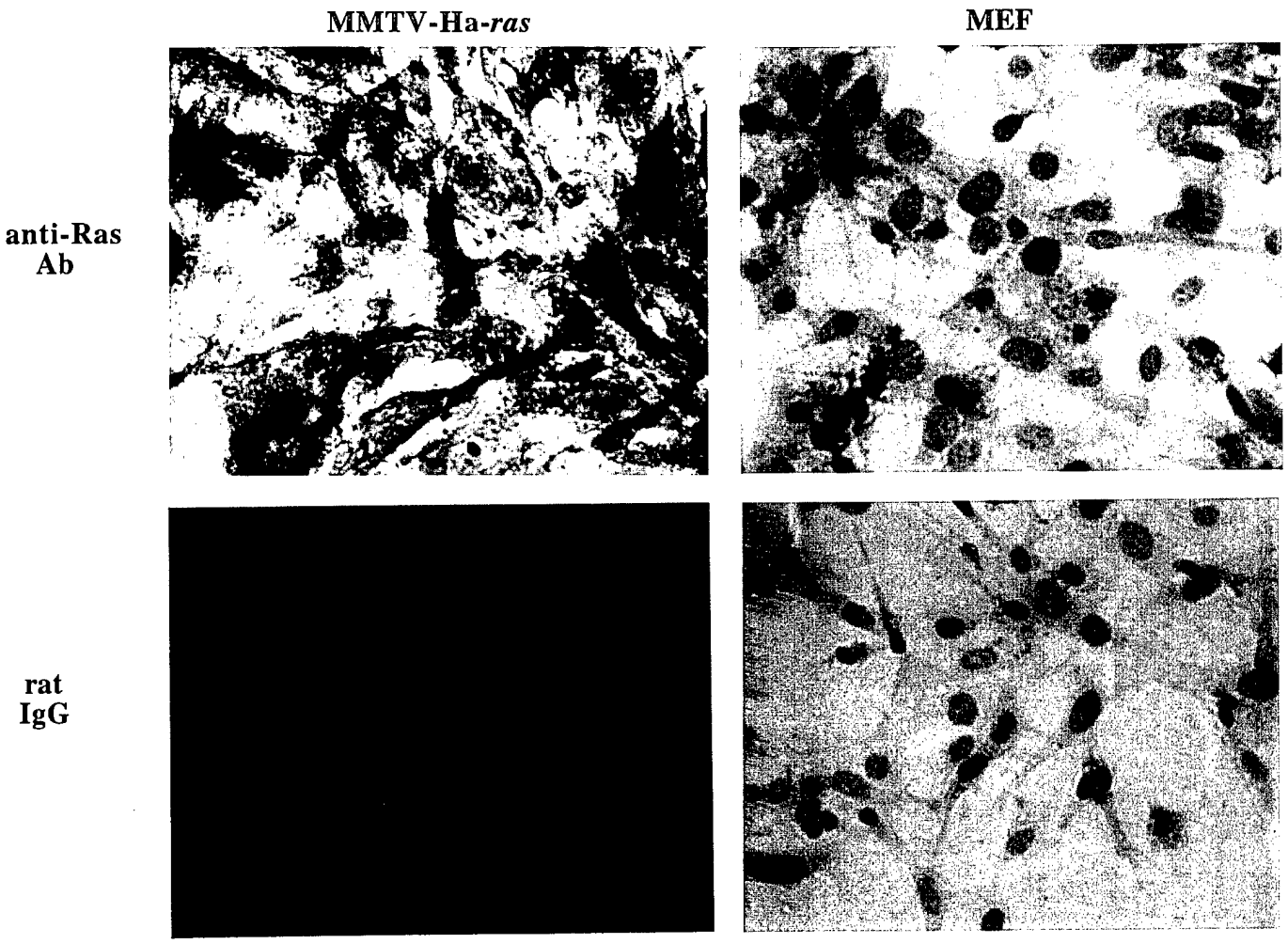

Figure 1. MMTV-Ha-*ras* mammary tumor cells (passage 5) or mouse embryo fibroblasts were plated and grown overnight on glass chambered slides (Lab-Tek II slides, Nalge Nunc). The following morning, cells were rinsed, fixed in 100% Methanol, blocked with BSA, and incubated with the anti-Ha-Ras primary antibody or rat IgG (control). The antibody was visualized with an anti-rat HRP-conjugated secondary antibody followed by DAB reaction.

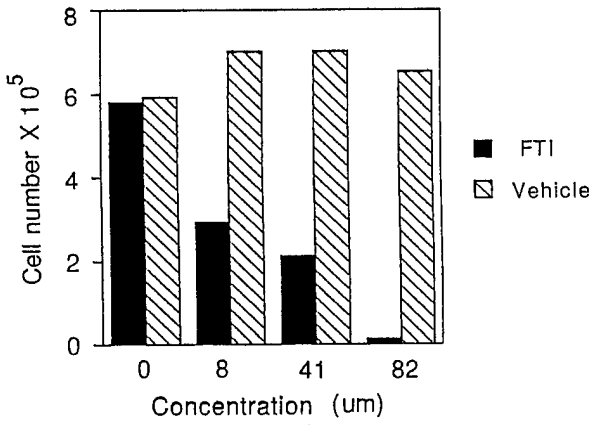

Figure 2. 1 X 10^4 MMTV-Ha-*ras* tumor-derived cells at passage 5 were plated in DMEM:F12 + 10% FBS, and 24 hrs later, either vehicle or varying concentrations of L-744,832 were added to the cultures. The cells were grown for 5 additional days, and were then trypsinized and counted.